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THE NATIONAL POULTRY IMPROVEMENT PLAN[1]

Prepared by specialists of the Bureau of Animal Industry, in cooperation with poultry leaders, poultry breeders, and members of the breeder and commercial hatchery industry

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The National Poultry Improvement Plan became operative July 1, 1935, with the approval of the Secretary of Agriculture and under the authority of an appropriation by Congress (Public No. 62, 74th Cong., H. R. 6718) for the Bureau of Animal Industry, United States Department of Agriculture, to be used in cooperation with State authorities in the administration of regulations for the improvement of poultry, poultry products, and hatcheries.

The acceptance of the plan is optional on the part of States and individual members of the industry within States. It is administered in each State by an official State agency cooperating with the Bureau of Animal Industry.

A list of the official State agencies engaged in the work of the plan may be obtained from this Bureau upon request. The names of members of the industry who are participating may be obtained from the official State agencies.

OBJECTIVES

The National Poultry Improvement Plan has been developed with a view to the establishment of the poultry-breeding industry upon

[1] The original edition of September 1935 and the revised editions of May 1936 and May 1937 were multigraphed. The text of this printed edition of the plan is the same as that of the revised multigraphed edition of May 1937.

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as sound a basis as possible. The adoption of the plan in all parts of the United States should bring about an improvement in the quality of hatching eggs, baby chicks, breeding stock, and market products. Such results should cause poultry production to be more efficient and thus aid in making the poultry enterprise more profitable.

The primary purpose of the plan is to identify, authoritatively, poultry-breeding stock, hatching eggs, and chicks with respect to quality by describing them in terms uniformly accepted in all parts of the country. Protection from unscrupulous competition is thereby afforded producers, and purchasers are able to buy with confidence.

Health is the foundation of successful husbandry since upon it depends successful production and reproduction. Constitutional vigor and sanitation provide the best insurance against ill health. Every poultryman and every hatcheryman, therefore, is under obligation to maintain the health of his laying and breeding stock and to keep his premises in the best possible state of sanitation.

Since the spread of the majority of the more important poultry diseases results from conditions of filth, it becomes evident that many disease conditions are amenable to sanitary measures.

GENERAL REGULATIONS

1. An official State agency is an agency such as the State department of agriculture, State college of agriculture, or other agency recognized officially by a State government. The official State agency shall direct, supervise, and be responsible for flock selection, testing for pullorum disease, and all other administrative work as provided for in the National Poultry Improvement Plan.

2. An official State inspector is an employee of an official State agency whose major duties comprise the official inspection of flocks, eggs, chicks, hatcheries, poultry premises, records, and methods employed in carrying on official State poultry improvement work.

3. All inspectors shall be authorized by the official State agency.

4. Flocks, eggs, chicks, and hatcheries shall be subject to inspection at least once a year by the properly constituted authority.

5. In all flocks tested for pullorum disease under this plan, the testing shall be done by properly qualified persons designated by the official State agency.

6. Poultry laying and breeding houses shall be kept clean and in good condition. The land in the immediate vicinity of the poultry laying and breeding houses shall be kept in sanitary condition.

7. Hatcheries and all incubator equipment shall be kept in strictly sanitary condition.

8. All eggs used in any stage of this plan shall be reasonably uniform in size and shape and sound in shell. In the case of all white-egg breeds U. S. Approved eggs shall be reasonably free from tints, and eggs of other U. S. breeding stages shall be free from tints.

9. The hatchery management shall maintain the identity of hatching eggs being incubated, including those which are being custom-hatched.

10. All chicks shall be normal and representative of the breed and variety and at hatching time shall weigh at least 8 pounds net per 100 chicks.

11. Delivery of chicks shall be made in approved types of chick boxes properly ventilated and securely tied, and chicks shall be shipped to reach the ultimate purchaser within the time limit prescribed by the United States Postal Regulations.

12. Chicks and hatching eggs produced under the rules and regulations of the National Poultry Improvement Plan shall lose their identity as of the terminology used in the plan when they are purchased for resale by any person, firm, or corporation not participating in the plan.

13. Chicks and hatching eggs of identical U. S. breeding stages and pullorum control and eradication classes may be exchanged or bought for resale by members of the industry who are participating in the same phases of the National Poultry Improvement Plan. Members of the industry may purchase for resale eggs or chicks of a higher stage or class than those which they produce, but such products may not be sold as of the higher stage or class.

14. Any person, firm, or corporation selling hatching eggs of the U. S. stages or classes, other than from the flock or flocks of such person, firm, or corporation, shall not sell hatching eggs from any flock or flocks other than those which are under the supervision of the National Poultry Improvement Plan. Adequate records shall be kept and shall at all times be available to the official State agencies concerned. These records shall consist of the names and addresses of all flock owners supplying hatching eggs, the number of females in each breeding flock, and the number of hatching eggs of each breed or variety sold to each purchaser.

15. With the approval of the official State agency, cross-bred chicks may be produced and sold by members operating under the National Poultry Improvement Plan, provided both males and females in the breeding flocks meet at least the minimum requirements of the U. S. Approved stage, including selection, leg-banding, and inspection. Such chicks shall not be designated as of any U. S. breeding stage.

16. When more than one hatchery, whether located within a State or in two or more States, is operated under the same name, ownership, or management, all the said hatcheries must comply with the regulations of the U. S. Approved or higher breeding stages before any one of them may offer for sale U. S. Approved chicks.

17. The use of the official terminology or any portion thereof of any particular breeding stage or pullorum control and eradication class of the National Poultry Improvement Plan in which members of the industry are participating shall be limited to hatching eggs, baby chicks, and breeding stock that meet at least the minimum requirements of that particular stage or class when produced by those under the jurisdiction of the official State agency.

18. Breeding stock and each bundle or package of hatching eggs and chicks sold or offered for sale shall be designated by official labels or tags defining the breeding stage and pullorum control and eradication class in accord with the regulations of the National Poultry Improvement Plan, except that the following shall not be sold or

offered for sale under official labels: (*a*) Breeding stock, hatching eggs, and chicks purchased for resale by a nonparticipating person, firm, or corporation (see General Regulation No. 12); (*b*) breeding stock, hatching eggs, and chicks consigned for sale; (*c*) sex-separated cockerel chicks to be sold for nonbreeding purposes.

19. All advertising must be in accord with the rules and regulations of the National Poultry Improvement Plan and the fair trade practice rules of the Federal Trade Commission for the baby-chick industry. Copies of proposed folders, catalogs, circulars, or other printed literature shall be submitted to and approved by the official State agency before being printed and distributed. Copies of such literature, after being printed, shall be filed with the official State agency. Copies of all publication advertising shall be mailed to the official State agency at or before the time these advertisements are submitted to the publisher or publishers.

20. All advertising mentioning blood test or blood testing shall specify the disease tested for and the method used in conducting the test and shall further state under what official supervision the test was conducted.

21. The term "tested" or "blood-tested" shall not be used in connection with disease eradication or control except as outlined in this plan.

22. The use of the word "accredited" to indicate breed improvement or disease eradication or control or its use in any other way is prohibited.

23. The selection of birds in a flock to qualify as U. S. Approved, U. S. Verified, or U. S. Certified by an official State inspector shall be regarded as one official flock inspection.

24. Males to be used in the U. S. Certified breeding stage may be dubbed prior to the time they reach 6 months of age, provided each bird has been officially examined and passed in respect to comb disqualifications; such birds must otherwise comply with all regulations pertaining to the qualifications of U. S. R. O. P.[2] males not earlier than 6 months of age. A record of the wing-band numbers of all U. S. R. O. P. male candidates dubbed shall be made and sent to the official State agency by each official State inspector within 10 days after the dubbing of each male in each flock is completed.

BREEDING STAGES

U. S. APPROVED (FIRST BREEDING STAGE)

U. S. Approved flocks shall meet the following requirements:

(1) Females shall be rigidly and thoroughly selected once each year for constitutional vigor and for egg production, such selected females to combine standard-bred and production qualities to a reasonably high degree; (2) males shall be selected especially for constitutional vigor and standard-bred qualities; (3) the selection of the flocks shall be approved by an official State inspector.

The method of production judging prescribed by the State college of agriculture or other properly constituted State agency shall serve as the basis for the selection of males and females. All birds to be

[2] Signifies U. S. Record of Performance. For brevity and convenience, this and similar terms are commonly abbreviated in poultry literature and in this publication.

used in the U. S. Approved flocks shall be selected by an official State inspector or by a flock-selecting agent, who shall be required to take a course of training in standard-bred and production judging prescribed by the State college of agriculture or other properly constituted State agency and shall be required to pass an examination and be authorized by the official State agency to do flock-selection work.

All birds selected for U. S. Approved flocks shall be banded at the time of selection with sealed and numbered leg bands secured through the official State agency. Birds rejected by the official State inspector or by the flock-selecting agent shall have their tails clipped, have their leg bands removed, and be kept separate from the breeding flocks. The selection of birds for a U. S. Approved flock shall be completed at least 20 days before any eggs are saved for hatching purposes, except that this 20-day interval shall not be required if all males found in the flock are approved.

Before the individual flock from which it is proposed to sell direct to purchaser U. S. Approved eggs or chicks may be recognized as a U. S. Approved flock, it shall be inspected and approved by an official State inspector. Before a hatchery may offer for sale U. S. Approved chicks of any one breed or variety, at least one-third of the hatchery flocks supplying eggs to the hatchery shall be inspected and approved by an official State inspector, and at least one-third of the flocks selected by each authorized selecting agent must be inspected and approved. The inspection of a flock by an official State inspector shall consist in the physical examination of a sufficient number of males and females to satisfy him that the flock is worthy of approval. All inspection work shall be completed before any eggs are saved for hatching.

No birds except those with official, sealed, and numbered leg bands shall be allowed in the breeding flocks, and no male birds shall be allowed in any other mature flock on the same premises except in U. S. Verified, U. S. Certified, or U. S. R. O. P. flocks.

Only one variety shall be kept on a farm unless, where more than one variety is kept, ample provision has been made to keep these varieties and their eggs separate.

U. S. Approved eggs shall come only from U. S. Approved flocks or a higher U. S. breeding stage, and shall weigh at least $1^{11}/_{12}$ ounces each.

U. S. Approved chicks shall be hatched only from U. S. Approved eggs and in U. S. Approved hatcheries.

U. S. Approved hatcheries shall be inspected by an official State inspector at least once during the hatching season. The management shall keep accurate records concerning the name and address of each flock owner, the number of eggs received from each flock owner, the name and address of each purchaser, the number, breed, and variety, and the date of shipment of all chicks. These records shall be open for official inspection at all times. Eggs received and chicks hatched shall be subject to inspection at all times. U. S. Approved hatcheries may not hatch for sale or sell hatching eggs and chicks other than those produced under the breeding stages of the National Poultry Improvement Plan, except as provided for in General Regulation No. 15. Only U. S. Approved and U. S. R. O. P. hatching eggs and chicks shall be hatched for sale or sold of the same variety. Hatching

eggs and chicks of U. S. Verified or U. S. Certified stages from other varieties may be hatched for sale or sold by a U. S. Approved hatchery.

U. S. VERIFIED (SECOND BREEDING STAGE)

U. S. Verified flocks shall fulfill all the requirements of the U. S. Approved flocks and shall be mated to males produced by flock matings of U. S. R. O. P. males to U. S. R. O. P. females. The males to be used in U. S. Verified flocks shall be wing-banded at hatching time with official, sealed wing bands in order that the cockerels reared may be identified at the time of inspection by the official State inspector.

The males and females used in U. S. Verified flocks shall be of at least qualifying standard weight as designated by the official State agency in cooperation with the State college of agriculture or other properly constituted State agency which gives the prescribed training in standard-bred and production judging. The birds shall be selected by an official State inspector or by a flock-selecting agent once each year, and all flocks shall be inspected by an official State inspector at least once during the hatching season.

U. S. Verified eggs shall come only from U. S. Verified flocks or a higher U. S. breeding stage and shall weigh at least $1^{11}/_{12}$ ounces each.

U. S. Verified chicks shall be hatched only from U. S. Verified eggs and in U. S. Verified hatcheries, except that a hatchery may produce and sell U. S. Verified chicks of one breed or variety and U. S. Approved of another breed or variety, in which case the hatchery shall be recognized as a U. S. Approved hatchery.

U. S. Verified hatcheries may not hatch for sale or sell hatching eggs and chicks other than U. S. Verified or higher breeding stages, except as provided for in General Regulation No. 15. Only U. S. Verified and U. S. R. O. P. hatching eggs and chicks shall be hatched for sale or sold of the same variety. U. S. Certified hatching eggs and chicks of other varieties may be hatched for sale or sold by a U. S. Verified hatchery. They shall fulfill all the requirements of U. S. Approved hatcheries and shall be inspected by an official State inspector at least twice during the hatching season.

U. S. CERTIFIED (THIRD BREEDING STAGE)

U. S. Certified flocks shall fulfill all the requirements of the U. S. Approved flocks and shall be mated to U. S. R. O. P. males. For the first 2 years of U. S: R. O. P. work by any breeder, the males for his own flock need not be U. S. R. O. P. males but must be individually pedigreed and of breeding which meets the qualifications for U. S. R. O. P. males. All such males may be used in subsequent breeding seasons if reinspected and certified by the official State agency. The males and females shall be of at least qualifying standard weight as designated by the official State agency in cooperation with the State college of agriculture or other properly constituted State agency which gives the prescribed training in standard-bred and production judging. The birds shall be selected by either an official State inspector or by a flock-selecting agent once each year, and all flocks shall be inspected by an official State inspector at least once during the hatching season.

U. S. Certified eggs shall come only from U. S. Certified flocks and shall weigh at least $1\frac{11}{12}$ ounces each and average at least 24 ounces per dozen.

U. S. Certified chicks shall be hatched only from U. S. Certified eggs and in U. S. Certified hatcheries, except that a hatchery may produce and sell U. S. Certified chicks of one breed or variety and U. S. Approved or U. S. Verified of another breed or variety, in which case the hatchery shall be recognized as of the lowest breeding stage in which chicks are produced.

U. S. Certified hatcheries may not hatch for sale or sell other than U. S. Certified hatching eggs and chicks, and U. S. R. O. P. hatching eggs and chicks, except as provided for in General Regulation No. 15. They shall fulfill all the requirements of U. S. Approved hatcheries and shall be inspected by an official State inspector at least twice during the hatching season.

U. S. RECORD OF PERFORMANCE (FOURTH BREEDING STAGE)

U. S. Record of Performance embraces records of egg production, egg weight, and body weight made on the breeder's premises under official supervision and similar records made at officially conducted egg-laying contests, when such records are passed upon by the official State inspector or official State supervisor and when the individual birds meet other U. S. R. O. P. requirements. The trap nests used in this work shall be of an approved type and shall be kept in perfect working order at all times. The poultry plant shall be open at all times for unannounced inspection by an official State inspector, and all egg-production records shall also be subject to inspection at all times. At the time of each inspection, the inspector shall have sole charge of the trap nests and of eggs laid. No females other than those entered in U. S. Record of Performance work shall be allowed in the pens.

U. S. R. O. P. eggs and chicks may be produced only on a U. S. R. O. P. breeder's premises except that with the approval of the official State agency (1) a U. S. R. O. P. breeder may have eggs hatched by any hatchery operating under the National Poultry Improvement Plan provided that the U. S. R. O. P. breeder does the pedigreeing of the chicks; and (2) a U. S. Approved, U. S. Verified, or U. S. Certified hatchery owner may produce U. S. R. O. P. chicks from purchased U. S. R. O. P. eggs for the use of his hatchery flock owners only, provided that proper records of purchases are sent to the official State agency and that an official State inspector does the wing banding of these chicks.

When U. S. R. O. P. eggs are transferred from one U. S. R. O. P. breeder to another U. S. R. O. P. breeder or hatcheryman for the purpose of hatching U. S. R. O. P. chicks, the conditions that shall govern such a transaction include the following:

(1) The U. S. R. O. P. breeder who sells U. S. R. O. P. hatching eggs must send a report in triplicate at the time the eggs are shipped, consisting of a list of the eggs sold, the pen and dam number of each egg, and the egg production, average egg weight, and body weight of each dam, to the official State agency of his State. If the eggs are shipped outside of the State, these records shall be attested to by the R. O. P. supervisor, who shall retain one copy, mail one copy to the

purchaser, and one to the official State agency of the State to which the eggs are shipped.

(2) Within 5 days after incubation has begun the purchasing U. S. R. O. P. breeder or hatcheryman must submit to his official State agency a list of the U. S. R. O. P. eggs bought.

(3) Within 5 days after hatching time the U. S. R. O. P. breeder or hatcheryman who purchased the eggs must send to his official State agency a list of the chicks hatched from each dam and their respective wing-band numbers.

When U. S. R. O. P. chicks, cockerels, or mature birds are sold or transferred by members of the industry participating in the National Poultry Improvement Plan, the individual making the sale must send a report in quadruplicate at the time these products are shipped, giving the number of birds sold; the number, egg production, average egg weight, and body weight of each dam; and the pen or leg-band number of the sire of each chick, cockerel, or mature bird, to the official State agency. If the chicks, cockerels, or mature birds are shipped outside of the State these records shall be attested to by the R. O. P. supervisor, who shall retain one copy, mail one copy to the purchaser, and one to the official State agency of the State to which the chicks, cockerels, or adult birds are shipped.

To be eligible for U. S. R. O. P. work all females shall be free from disqualifications and shall have been raised from U. S. R. O. P. or U. S. Certified flocks or other flocks of breeding satisfactory to the official State agency. Eligibility shall be confined to any standard breed or variety or any other breed or variety recognized by the United States Record of Performance Federation.

All birds trap-nested on the premises of a U. S. R. O. P. breeder longer than 60 days shall be considered as entered under U. S. R. O. P. supervision with the following four exceptions: (1) All qualified U. S. R. O. P. hens may be trap-nested; (2) a U. S. R. O. P. breeder may trap-nest certain birds for as long a period as 6 months for the purpose of obtaining progeny-test records on particular families; this additional private trapping is to be conducted only with the knowledge and consent of the official State agency and the trap-nest records on these birds must at all times be open and available to the supervisor or his inspector; (3) he may, with the approval of the official State agency, trap-nest any other breed or variety which he is developing as a basis for future U. S. R. O. P. work; and (4) with the approval of the official State agency, hens in their second or subsequent production years may be privately trapped to complete lifetime egg records that were started before U. S. R. O. P. work was undertaken. Such birds shall not be trap-nested in pens with U. S. R. O. P. candidates.

All flocks shall be inspected at least seven times a year. One or more consecutive days of inspection by the R. O. P. inspector shall represent a single inspection. In the event of an outbreak of contagious disease in a poultry plant where U. S. R. O. P. work is carried on, inspection may be temporarily suspended.

R. O. P. supervisor.—The R. O. P. supervisor shall represent the official State agency supervising U. S. R. O. P. work, and his supervision shall be of such nature that he shall vouch for the accuracy of all U. S. R. O. P. records made by authorized U. S. R.

O. P. breeders in his jurisdiction. He shall keep on file in his office (1) a record of all qualified males, with at least one-generation pedigree showing egg records and egg weights of the female ancestors, and (2) a record of all qualified females with their egg records and egg weights for their first year of production. He shall also keep on file a record of all U. S. R. O. P. matings and all U. S. R. O. P. chicks wing-banded per each breeding female with one-generation pedigree. If necessary, because of lack of storage space, these records may be destroyed when 5 years old.

The R. O. P. supervisor, in publishing a summary of the U. S. R. O. P. work under his supervision each year, shall include the following information for each flock in his U. S. Record of Performance report:

1. Total number of pullets of that particular breed on the farm.
2. Original number of pullets trapped for entry in U. S. R. O. P.
3. Number meeting the U. S. R. O. P. requirements.
4. Percentage meeting the U. S. R. O. P. requirements.
5 Number of birds qualifying which lay from 200 to 249 eggs.
6. Number of birds qualifying which lay 250 or more eggs.
7. Average egg weight of the qualifying birds.
8. Average body weight of the qualifying birds.
9. Average production of the qualifying birds.

The R. O. P. supervisor may bring charges against any U. S. R. O. P. breeder for falsifying records, unethical conduct in his business methods, or for any other cause which he may consider detrimental to U. S. R. O. P. work. Upon such charges being proved in a fair hearing the offending breeder must be reprimanded or suspended, according to the nature of the offense.

R. O. P. inspector.—The R. O. P. inspector shall work under the direction of the R. O. P. supervisor. It shall be his duty to visit and inspect the work of each U. S. R. O. P. breeder at least seven times each year, his visit to be unannounced and so timed that each season of the year shall be represented in his reports. On each visit he shall do the trap-nesting for the day and shall record all eggs and egg weights of all eggs laid by each individual hen entered in U. S. R. O. P. During the breeding season he shall examine all hens in U. S. R. O. P. matings to see that credit for eggs laid is not being given to hens apparently out of production and to see that the birds which constitute these matings are listed with the supervisor. He shall compare the number of eggs being incubated from each hen with the number she is credited with having laid during the corresponding period, and shall have authority to test these eggs for fertility. When the hatch is reported he shall compare his findings with the number of chicks reported hatched and wing-banded. He shall be at all times courteous to breeders but shall be on the alert to discover errors in records or violations of rules, intentional or otherwise. He shall immediately report to the supervisor any suspicious circumstances or appearance of fraud in the records or business dealings of a U. S. R. O. P. breeder.

U. S. R. O. P. breeder.—A U. S. R. O. P. breeder is one operating a poultry-breeding plant under official State supervision. He shall have all his mated birds and hatchery under the supervision of the official State agency. His flock or flocks must consist of purebred

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poultry of a recognized breed and must be trap-nested at regular intervals every day throughout the year. He shall send to his supervisor, not later than the tenth day of each month, a complete report of all U. S. R. O. P. egg records and egg weights for the previous month. His egg, pedigree, and sales records shall at all times be open to examination by the supervisor or inspector.

U. S. R. O. P. females.—Pullets shall have laid 200 or more eggs during the first laying year, which, in the case of birds on the breeders' premises, shall mean 365 days from the time of laying the first egg in a trap nest, provided that all first-year records shall end by November 30, except that pullets entered in U. S. R. O. P. after December 1 may be allowed to complete a full year's record, and such records that enable pullets to qualify for U. S. R. O. P. shall be included in the following year's report. In the case of contest pullets, the first laying year shall mean the duration of the official contest year.

In order to qualify for U. S. R. O. P. the eggs of each pullet must average at least 24 ounces per dozen. For 3 successive days each month, beginning with the month of January and continuing through the remainder of the birds' laying year, the breeder shall record the weight of *each egg laid*. Yearling and older hens entered for U. S. R. O. P. qualification shall have laid 200 or more eggs during the trap-nest year, which shall mean 365 days from the date of the first egg laid in a trap nest. The eggs of each yearling or older hen entered for U. S. R. O. P. qualification shall attain an average weight of 25 ounces per dozen. The inspector shall weigh all eggs on each day's official inspection of the U. S. R. O. P. candidates, beginning in January and continuing throughout the year. An average of all egg weights taken by the breeder and the inspector for each female during her first trap-nest year shall be her official average egg weight.

All females of white-egg-laying varieties laying tinted eggs shall be removed from U. S. R. O. P. flocks. All females laying eggs of undesirable shape shall be removed from U. S. R. O. P. flocks.

Any bird showing a disqualification may be removed from a U. S. R. O. P. flock by an official State inspector at any time.

All females shall be of at least qualifying body weight for hens for each breed or variety as designated in the American Standard of Perfection, allowance being made for the standard weight tolerance except that 3 pounds' tolerance shall be allowed for Plymouth Rocks, Jersey Giants, and breeds in the English and Asiatic classes. The weights of each female entered in U. S. R. O. P. shall be taken by the R. O. P. inspector at least twice between January 1 and August 1, and the average of these weights shall be used for purposes of qualifying for U. S. R. O. P. All birds qualifying as U. S. R. O. P. females shall be banded with a U. S. R. O. P. official, sealed, and numbered leg band as soon as possible after qualifying.

U. S. R. O. P. males.—Males produced from U. S. R. O. P. chicks shall qualify as U. S. R. O. P. males provided they shall have passed the final individual inspection of an official State inspector not earlier than at 6 months of age. Each male passed by an inspector shall possess strong constitutional vigor and shall be a good representative of the breed or variety, shall be free from standard disqualifications, and shall be banded with a U. S. R. O. P. official, sealed,

and numbered leg band when passed by the inspector. At the time of inspection and banding, the inspector shall make a complete record of the wing- and leg-band numbers of each male banded.

U. S. R. O. P. matings shall comprise only U. S. R. O. P. females mated to any U. S. R. O. P. male from a U. S. R. O. M female or a U. S. R. O. P. female with a minimum record of 225 eggs. The breeder may place in the U. S. R. O. P. breeding pen females that are not U. S. R. O. P., provided that he does not sell pedigreed eggs, chicks, or stock from these non-U. S. R. O. P. females. The males shall be of at least qualifying weight as outlined in the American Standard of Perfection. For the first year of U. S. R. O. P. breeding work by any breeder the male mated with U. S. R. O. P females need not be a U. S. R. O. P. male provided he is of equal pedigree. No eggs or progeny from such a mating shall be sold as U. S. R. O. P. products, except that male offspring produced during the first breeding year may be permitted to head U. S. Certified flocks in the State where produced.

Only one male shall be allowed in a single breeding pen at any one time, and the date of entry and removal of males shall be properly recorded.

The females shall be trap-nested throughout the breeding season, and each egg shall be numbered with the U. S. R. O. P. number of the female that laid it and the number of the mating. For each female, an accurate record shall be kept of all eggs laid during the breeding season, the number of eggs sold, the number of eggs incubated, and the total number of good chicks hatched and banded. Upon request, eggs that do not hatch shall be held for the inspector.

If the eggs are to be incubated to produce U. S. R. O. P. chicks, a report must be made of the number of eggs from each hen, with her pen number, a copy of which must be sent to the supervisor within 5 days after incubation of the eggs has begun.

All U. S. R. O. P. breeding pens shall be inspected at least twice during the breeding season.

U. S. R. O. P. eggs offered for sale or used for hatching shall come only from U. S. R. O. P. matings and shall weigh at least 2 ounces each and 25 ounces or more per dozen. Each egg shall bear the U. S. R. O. P. number of the hen that laid it and the number of the breeding pen.

U. S. R. O. P. chicks shall come only from U. S. R. O. P. eggs. The chicks from each female shall be hatched separately in pedigree baskets or sacks and shall be banded individually with official, sealed, and numbered wing bands at the time of removal from pedigree baskets or sacks. An accurate record of all chicks banded shall be furnished the R. O. P. supervisor within 5 days after the date of hatch. The chicks shall be subject to inspection at any time. The bands from dead chicks shall be retained for the information of the inspector and shall be furnished to him upon request.

Fraud.—Evidence of fraud in connection with any form of U. S. R. O. P. work when established by the official State agency after a thorough investigation and hearing shall bar from further U. S. R. O. P. work, for a period of at least 2 years, the person or persons found to have committed the fraud.

U. S. REGISTER OF MERIT (FIFTH BREEDING STAGE)

U. S. R. O. M. male.—A U. S. R. O. M. male is a U. S. R. O. P. male which, when mated to U. S. R. O. P. or U. S. R. O. M. females, has at least one-third of his daughters entered in U. S. R. O. P. qualify for U. S. R. O. P. and a minimum of 20 that qualify.

U. S. R. O. M. female.—A U. S. R. O. M. female is a U. S. R. O. P. female out of a U. S. R. O. P. or U. S. R. O. M. female, which, when mated to a U. S. R. O. P. or U. S. R. O. M. male in an individual pedigree mating, has at least one-third of her daughters entered in U. S. R. O. P. qualify for U. S. R. O. P. and a minimum of four that qualify. U. S. R. O. P. candidates, progeny of a U. S. R. O. P. mating, which later qualify as U. S. R. O. P. females, shall be permitted to qualify for U. S. R. O. M. through their female progeny, provided that the candidates are mated to a U. S. R. O. P. male out of a U. S. R. O. P. female with a minimum record of 225 eggs and that these matings are handled in all other respects as a U. S. R. O. P. mating except that the wing bands used cannot be U. S. R. O. P. wing bands.

U. S. REGISTER OF MERIT MATINGS

A. A double-star U. S. R. O. M. mating is a mating of a U. S. R. O. M. male and a U. S. R. O. M. female.

B. A single-star U. S. R. O. M. mating is a mating consisting of any of the five following combinations:

1. U. S. R. O. M. male × U. S. R. O. P. daughters out of a double-star U. S. R. O. M. mating.
2. U. S. R. O. M. male × U. S. R. O. P. females out of a U. S. R. O. M. female.
3. U. S. R. O. P. male out of double-star U. S. R. O. M. mating × U. S. R. O. M. females.
4. U. S. R. O. P. male out of a U. S. R. O. M. female × U. S. R. O. M. females.
5. U. S. R. O. P. males, as in nos. 3 or 4, × females as in no. 2.

PULLORUM CONTROL AND ERADICATION

1. (*a*) Matters relating to disease shall be under the direction of the livestock sanitary authorities or officials of the State college of agriculture or similarly authorized State agency of the respective States in cooperation with the United States Bureau of Animal Industry.

(*b*) In order to qualify as a U. S. Pullorum-Tested flock, the testing of birds for carriers of the pullorum organism shall be done by a livestock sanitary authority, an official of the State college of agriculture or similarly authorized State employee, or a pullorum-testing agent, who shall be required to take a course of training in pullorum testing prescribed by the livestock sanitary authorities or officials of the State college of agriculture and shall be required to pass an examination and be authorized by the official State agency to do pullorum-testing work. The livestock sanitary authorities, officials of the State college of agriculture, or similarly authorized State employees, under whose supervision the pullorum-testing work is carried on, shall assume responsibility for the accuracy of the testing work done by the pullorum-testing agents.

(*c*) In order to qualify as a U. S. Pullorum-Passed or U. S. Pullorum-Clean flock, the testing of birds for carriers of the pullorum organism shall be done by a livestock sanitary authority, an official of the State college of agriculture, or similarly authorized State employee.

2. In the control of pullorum disease on any farm or poultry plant, all chickens over 4 months of age to be used as breeders in the case of flocks qualifying as U. S. Pullorum-Tested and U. S. Pullorum-Clean and all chickens over 4 months of age in case of flocks qualifying as U. S. Pullorum-Passed, must be tested for pullorum disease by means of some officially approved method, and reactors must be removed from the premises upon the completion of the test, and the premises must be immediately carefully cleaned and disinfected to the satisfaction of the official State agency.

3. The official test for pullorum disease in the National Poultry Improvement Plan shall be one of the following agglutination tests:

(*a*) The standard tube agglutination test, as described in the Proceedings of the United States Live Stock Sanitary Association, November 30 to December 2, 1932, pages 487 to 491, or

(*b*) The stained-antigen, rapid, whole-blood test, as described by Schaffer, MacDonald, Hall, and Bunyea, in the Journal of the American Veterinary Medical Association, volume 79 (n. s. 32), No. 2, pages 236–240, 1931, and covered by U. S. Patent 1,816,026, or

(*c*) The rapid serum test, as described by Runnells, Coon, Farley, and Thorp, Journal of the American Veterinary Medical Association, volume 70 (n. s. 23), No. 5, pages 660–662, 1927.

(For convenience of reference the essential features of the three tests are reprinted in the appendix of this plan on p. 19.)

4. The stained antigen used in the whole-blood testing [see 3, (*b*)] for pullorum disease shall be approved by the United States Department of Agriculture.

5. In the event that subsequent tests of U. S. Pullorum-Passed or U. S. Pullorum-Clean flocks reveal reactors, the official State agency shall have the right to request the owner to submit all of the reactors for autopsy and bacteriological examination, and in case such autopsy and bacteriological examination fail to reveal any evidence of pullorum infection the flock shall be considered to have been free of pullorum disease.

6. No other eggs shall be incubated in the same incubator or in the same room as eggs from U. S. Pullorum-Tested, U. S. Pullorum-Passed, or U. S. Pullorum-Clean flocks. Eggs from U. S. Pullorum-Tested flocks shall not be incubated in the same incubator or in the same room as eggs from U. S. Pullorum-Passed or U. S. Pullorum-Clean flocks. No other chicks shall be hatched in the same incubator or in the same room or brooded in the same room as chicks from U. S. Pullorum-Tested, U. S. Pullorum-Passed, or U. S. Pullorum-Clean flocks. Chicks from U. S. Pullorum-Tested flocks shall not be hatched in the same incubator or in the same room as chicks from U. S. Pullorum-Passed or U. S. Pullorum-Clean flocks.

In separating a room for the use of two or more incubators, in order to comply with this provision, a tight partition is absolutely necessary and must be provided. Outside ventilation and an outside entry for each room are required. Any door or opening between the two rooms must be sealed when chicks are being hatched, packed, or

stored in the room where eggs from non-U. S. Pullorum-Tested flocks are hatching. All incubators used for hatching U. S. Pullorum-Tested, U. S. Pullorum-Passed, or U. S. Pullorum-Clean chicks must be thoroughly cleaned and disinfected by spraying or fumigation after each hatch, by means of an officially recognized method.

7. In the case of U. S. Pullorum-Passed and U. S. Pullorum-Clean flocks, all birds which are returned to the premises from poultry exhibits or egg-laying contests or which have been removed from the premises for any purpose whatsoever, shall be quarantined for at least 30 days and officially tested once at the time of their return and again at least 30 days later, and any reactors shall be removed immediately after each test.

8. U. S. Pullorum-Tested, U. S. Pullorum-Passed, or U. S. Pullorum-Clean classes of chicks, flocks, and hatcheries may be attained, produced, advertised, and sold by any hatchery or poultry breeder complying with the requirements outlined in this plan. When these classes are attained, produced, advertised, or sold by breeders or hatcheries operating as U. S. Approved, U. S. Verified, U. S. Certified, or U. S. R. O. P. in accordance with this plan, the proper description of such flocks, hatcheries, and chicks shall be U. S. Approved, Pullorum-Tested; U. S. Approved, Pullorum-Passed; U. S. Approved, Pullorum-Clean; U. S. Verified, Pullorum-Tested; U. S. Verified, Pullorum-Passed; U. S. Verified, Pullorum-Clean; U. S. Certified, Pullorum-Tested; U. S. Certified, Pullorum-Passed; U. S. Certified, Pullorum-Clean; U. S. R. O. P., Pullorum-Tested; U. S. R. O. P., Pullorum-Passed; or U. S. R. O. P., Pullorum-Clean.

U. S. PULLORUM-TESTED CLASS

U. S. Pullorum-Tested flocks.—Flocks, any members of which are used as breeders, which when tested for pullorum disease under the supervision of an official State agency contain fewer than 10 percent of reactors, the last test being made within 12 months immediately preceding the date of sale of hatching eggs or chicks from such flocks. All indicated carriers of pullorum disease shall have been removed from the premises upon completion of the test and disposed of in a manner satisfactory to the official State agency. All birds remaining in the flock shall be properly leg-banded. Individual birds introduced into U. S. Pullorum-Tested flocks shall have passed, within 12 months, a negative test for pullorum disease.

A flock containing 10 percent or more of reactors on the first test, upon being retested at intervals of not less than 30 days and all reactors removed after each test until the percentage of reactors is less than 10, may qualify as a U. S. Pullorum-Tested flock.

U. S. Pullorum-Tested hatchery.—A hatchery operating under the supervision of an official State agency and hatching eggs only from U. S. Pullorum-Tested, U. S. Pullorum-Passed, or U. S. Pullorum-Clean flocks, except custom-hatching, which may be carried on as prescribed in item 6, page 13.

U. S. Pullorum-Tested chicks.—Chicks hatched from eggs produced by U. S. Pullorum-Tested, U. S. Pullorum-Passed, or U. S. Pullorum-Clean flocks and hatched in a U. S. Pullorum-Tested hatchery.

U. S. Pullorum-Tested eggs.—Eggs from U. S. Pullorum-Tested flocks.

U. S. PULLORUM-PASSED CLASS

U. S. Pullorum-Passed flocks.—Flocks which, when tested for pullorum disease under the supervision of an official State agency, contain no reactors, the last test having been made within the testing year immediately preceding the date of sale of hatching eggs or chicks from such flocks. All pullorum tests of flocks of this class, or flocks that are candidates for this class, must be reported to the supervising official State agency, and reactors occurring in unofficial tests shall be considered on the same basis as any reactors in official tests.

Birds may not be added to U. S. Pullorum-Passed flocks except after the approval of the official State inspector and then only from U. S. Pullorum-Passed or U. S. Pullorum-Clean flocks.

U. S. Pullorum-Passed hatchery.—A hatchery operating under the rules of an official State agency and hatching eggs or brooding chicks from only U. S. Pullorum-Passed or U. S. Pullorum-Clean flocks.

U. S. Pullorum-Passed chicks.—Chicks hatched from eggs produced by U. S. Pullorum-Passed or U. S. Pullorum-Clean flocks and hatched in a U. S. Pullorum-Passed hatchery.

U. S. Pullorum-Passed eggs.—Eggs from U. S. Pullorum-Passed flocks.

U. S. PULLORUM-CLEAN CLASS

U. S. Pullorum-Clean flocks.—Flocks, any members of which are used as breeders which, when tested for pullorum disease under supervision of an official State agency, contain no reactors in two consecutive tests not less than 6 months apart, the last test being made within the testing year immediately preceding the date of sale of hatching eggs or chicks from such flocks; provided that the first one of these two consecutive tests shall have met all the provisions of a U. S. Pullorum-Passed flock. Once a flock is established as U. S. Pullorum-Clean it remains so as long as no reactors are found in the official annual test of birds used as breeders. When one reactor or not more than 0.5 percent of a flock and in any case not more than six birds in any flock which in the previous year or years had a U. S. Pullorum-Clean rating is found to be positive on any test for pullorum disease, a retest of all birds to be used as breeders may be made at the discretion of the official State agency not earlier than 30 days or later than 6 weeks after the first test. If the retest reveals no reactors the flock may again be declared to be U. S. Pullorum-Clean. All pullorum tests of flocks of this class or flocks that are candidates for this class must be reported to the supervising official State agency, and reactors occurring in unofficial tests shall be considered on the same basis as any reactors in official tests.

A flock developed exclusively from purchased hatching eggs produced by a U. S. Pullorum-Clean flock and hatched in a U. S. Pullorum-Clean hatchery may be recognized as a U. S. Pullorum-Clean flock in one annual test conducted under the supervision of an official State agency if no reactors are found.

Birds may not be added to U. S. Pullorum-Clean flocks except after the approval of the official State agency and then only from U. S.

Pullorum-Clean flocks. The number of birds and the name and address of the person from whom the purchase is to be made shall be furnished the official State agency when application is made for birds to be added to the original flock. In moving birds from one farm to another due precaution should be taken to use clean, sanitary coops.

U. S. Pullorum-Clean hatchery.—A hatchery operating under the rules of an official State agency and hatching eggs or brooding chicks from only U. S. Pullorum-Clean flocks.

U. S. Pullorum-Clean chicks.—Chicks hatched from eggs produced by U. S. Pullorum-Clean flocks and hatched in a U. S. Pullorum-Clean hatchery.

U. S. Pullorum-Clean eggs.—Eggs from U. S. Pullorum-Clean flocks.

SUMMARY OF THE NATIONAL POULTRY IMPROVEMENT PLAN

OBJECTIVES OF THE PLAN

To improve the production and breeding qualities of poultry.

To reduce the mortality of chicks from pullorum disease.

To identify authoritatively breeding stock, hatching eggs, and chicks, with respect to quality, by describing them in terms uniformly accepted in all parts of the United States.

To serve as a means through which scientific research may be applied for the improvement of poultry and poultry products.

GENERAL FEATURES OF THE PLAN

The plan is Nation-wide in scope. Participation is optional on the part of the States and members of the industry within the States.

Five progressive breeding stages and three progressive pullorum-control classes are provided.

Minimum requirements are established for each breeding stage and each pullorum-control class.

The breeding and the pullorum-control work may be carried on independently of each other if the official State agency in question approves. However, it is recommended that both phases be followed simultaneously.

The plan is administered in each State by an official State agency in cooperation with the Federal Bureau of Animal Industry.

Official inspections are made by official State inspectors who are employees of official State agencies.

To meet the requirements of the plan, all persons who do flock-selecting and pullorum-testing work must be authorized by the official State agency.

Flock-selecting agents and pullorum-testing agents are individuals who have qualified under the supervision of an official State agency to perform flock-selecting and pullorum-testing work respectively.

Only standard-bred birds are eligible to qualify as breeding stock.

All birds used for breeders are to be selected annually and are to be identified with sealed leg bands obtained through the official State agency.

The premises and the equipment of participating flock owners and hatcherymen are to be kept in sanitary condition.

Under certain restrictions hatchery products may be exchanged or bought for resale by those who are participating in the plan.

The official terminology of the plan is limited to those who are participating in its provisions. When both the breeding and the pullorum-testing phases of the program are followed a combination of terms with the prefix U. S. is provided.

Breeding stock and hatchery products offered for sale are to be designated by official labels or tags.

Accurate records are to be kept by participating breeders and hatcherymen concerning certain details of their operations.

All advertising is to be submitted to and approved by the official State agency.

BREEDING STAGES OF THE PLAN

U. S. APPROVED BREEDING STAGE

This breeding stage provides for U. S. Approved flocks, U. S. Approved eggs, U. S. Approved chicks, and U. S. Approved hatcheries.

Both males and females are to be selected for constitutional vigor and for standard-bred and production qualities and banded by either State inspectors or flock-selecting agents.

At least one-third of the flocks supplying eggs to a hatchery are to be inspected and approved by a State inspector before any eggs are saved for hatching purposes if and when the breeders are selected and banded by flock-selecting agents.

Hatching eggs are to weigh at least $1\frac{11}{12}$ ounces each.

Each hatchery is to be inspected once or more often during the hatching season.

U. S. VERIFIED BREEDING STAGE

This breeding stage provides for U. S. Verified flocks, U. S. Verified eggs, U. S. Verified chicks, and U. S. Verified hatcheries.

The males are to be from U. S. Record of Performance flock matings.

The females may be selected by either State inspectors or flock-selecting agents.

All flocks are to be inspected and verified once or more often during the hatching season by a State inspector.

Hatching eggs are to weigh at least $1\frac{11}{12}$ ounces each.

Each hatchery is to be inspected twice or more often during the hatching season by an official State inspector.

U. S. CERTIFIED BREEDING STAGE

This breeding stage provides for U. S. Certified flocks, U. S. Certified eggs, U. S. Certified chicks, and U. S. Certified hatcheries.

The males are to be from U. S. Record of Performance individual matings.

The females may be selected by either State inspectors or flock-selecting agents.

All flocks are to be inspected and certified once or more often during the hatching season by a State inspector.

Hatching eggs are to weigh at least $1\frac{11}{12}$ ounces each and average at least 24 ounces per dozen.

Each hatchery is to be inspected by an official State inspector at least twice during the hatching season.

U. S. RECORD OF PERFORMANCE BREEDING STAGE

This breeding stage provides for U. S. R. O. P. flocks, U. S. R. O. P. eggs, U. S. R. O. P. chicks, and U. S. R. O. P. breeding stock.

The females are to be trap-nested and, to qualify, must lay 200 or more eggs in 1 year. Detailed records are to be kept on all operations.

The males are to come from hens which have laid 225 or more eggs in 1 year.

Both males and females are to be free of standard disqualifications and to comply with standard weight requirements.

Eggs during the first year of laying are to weigh on an average at least 24 ounces per dozen and thereafter average 25 ounces per dozen.

All flocks are to be inspected by a State inspector at least seven times during the year.

All chicks are to be individually pedigreed and wing-banded at hatching time.

U. S. REGISTER OF MERIT BREEDING STAGE

In this most advanced breeding stage recognition is given only to U. S. R. O. P. males and U. S. R. O. P. females whose progeny excel in performance.

To qualify in this stage a U. S. R. O. P. male must have at least one-third of his daughters qualify in U. S. R. O. P. and a minimum of 20 that qualify.

A U. S. R. O. P. female must have at least one-third of her daughters qualify in U. S. R. O. P. and a minimum of four that qualify in order to qualify in this breeding stage.

PULLORUM CONTROL AND ERADICATION

METHOD OF TESTING

Any one of the following methods may be used in testing chickens for pullorum disease:

(*a*) The standard tube agglutination test.
(*b*) The stained-antigen, rapid, whole-blood test.
(*c*) The rapid serum test.

U. S. PULLORUM-TESTED CLASS

All chickens over 4 months of age to be used as breeders are to be tested by a State inspector or a testing agent and the reactors removed from the premises. Each flock must contain fewer than 10 percent of reactors.

The testing work must be done within the 12 months immediately preceding the date of sale of hatching eggs or chicks.

All chickens introduced into the breeding flock must likewise have been tested and be negative to the tests.

Eggs from nontested flocks are not to be incubated in the same machine or in the same room with eggs from U. S. Pullorum-Tested flocks.

Chicks from nontested flocks are not to be hatched or brooded in the same machine or in the same room with chicks from U. S. Pullorum-Tested flocks.

U. S. PULLORUM-PASSED CLASS

All chickens over 4 months of age are to be tested by a livestock sanitary authority, an official of the college of agriculture, or an authorized State employee.

The flock must not contain reactors on the last test made within the testing year immediately preceding the date of sale of hatching eggs, chicks, or breeding stock.

Birds introduced into the flock must be from U. S. Pullorum-Passed and/or U. S. Pullorum-Clean flocks.

No eggs other than from U. S. Pullorum-Clean flocks are to be incubated in the same incubator or in the same room with eggs from U. S. Pullorum-Passed flocks.

No chicks other than from U. S. Pullorum-Clean flocks are to be hatched or brooded in the same machine or in the same room as chicks from U. S. Pullorum-Passed flocks.

U. S PULLORUM-CLEAN CLASS

All birds over 4 months of age to be used as breeders are to be tested annually by a livestock sanitary authority, an official of the college of agriculture, or an authorized State employee.

The flock must not contain reactors in two consecutive tests not less than 6 months apart, the last test being made within the testing year immediately preceding the date of sale of hatching eggs, chicks, or breeding stock.

Birds introduced into the flock must be from only U. S. Pullorum-Clean flocks.

Eggs from nontested, pullorum-tested, or pullorum-passed flocks are not to be incubated in the same incubator or in the same room with eggs from pullorum-clean flocks.

Chicks from other flocks are not to be hatched or brooded in the same machine or in the same room as chicks from U. S. Pullorum-Clean flocks.

APPENDIX

OFFICIAL TESTING FOR PULLORUM DISEASE

For the benefit of active workers in laboratory and field, the essential portions of the original articles describing the three recognized methods of testing are herein reprinted verbatim.[3]

THE STANDARD TUBE AGGLUTINATION TEST

(Thirty-sixth Annual Meeting of the United States Live Stock Sanitary Association; Report of the Conference of Official Research Workers in Animal Diseases, II. Serological Diagnosis of Pullorum Disease; Jour. Amer. Vet. Med. Assoc. 82 (n. s. 35): 488–490, 1933).

Collection and delivery of blood samples.—The blood samples shall be taken by properly qualified and authorized persons only, and in containers provided by the diagnostic laboratory or other authorized agent. The containers should be stout-walled test-tubes, preferably ⅜ inch by 3 inches, without lip, or small

[3] By permission of the editor of the Journal of the American Veterinary Medical Association.

well-selected medicine vials, which have been thoroughly cleaned, and dried in a hot-air drying-oven. The tubes are accompanied by selected cork stoppers which, unless new, have been washed and thoroughly boiled or scalded, and dried. All birds must be officially leg-banded with bands supplied by the control agency.

The blood is procured by making a small incision in the large median wing vein, with a small sharp lancet, and allowing the blood to run into the tube, or by the use of a small syringe (with 20- or 21-gage needle) which is properly cleansed between bleedings with physiological saline solution. From one and one-half to two cubic centimeters of blood should be collected. The tubes are corked and laid on their sides in order to allow the blood to solidify in a long slant. After the tubes have completely clotted, they are packed and shipped by mail (special delivery), rapid express, or by messenger, to the laboratory. All labeling must be clear and permanent, and may be done with a suitable pencil on etched portions of the tubes, or by means of fast-gum labels.

The blood samples must reach the laboratory in a fresh and unhemolyzed condition. Hemolyzed samples should be rejected. It is imperative, therefore, to cool the tubes immediately after slanting and clotting, and unless they reach the laboratory within a few hours, to pack them with ice in special containers, or by some other cooling system which will insure their preservation during transportation. In severe cold seasons, extreme precaution must be exercised to prevent freezing and consequent laking. The samples must be placed in cold (5° to 10° C.) storage immediately upon arrival at the laboratory.

Preparation of antigen.—The antigen shall consist of three representative strains of *S. pullorum* which are of known, high agglutinability, but are not sensitive to negative and to nonspecific sera. They are grown on agar having the following composition:

Water	1,000 cc.
Difco beef extract	4 gm (0.4 percent).
Difco Bacto-peptone	10 gm (1.0 percent).
Dry granular agar (Difco)	20 gm (2.0 percent).

Reaction-pH 6.8 to 7.2.

Large 1-inch test tubes, kolle flasks or Blake bottles are streaked liberally over the entire agar surface with inoculum from *48-hour, slant-agar cultures* prepared from the stock cultures of the selected strains.[4] Frequent resort to the stock cultures is highly desirable, in order to maintain the purity of the antigen, and to allow *long rest intervals for the organisms.* A second intermediate culture may be necessary at times to furnish luxuriant growths for the final transfers. The antigen-growing tubes or bottles are incubated 48 hours at 37° C. and the surface growth washed off with sufficient phenolized (0.5 percent) saline (0.85 percent) solution to make a heavy suspension or very light paste. The washings are filtered through lightly packed absorbent cotton placed in the apex of the funnel. The washings of the three separate strains are now combined in equal volume-density and stored in the refrigerator (5° to 10° C.) in tightly stoppered bottles.

The dilute antigen to be used in the routine testing is prepared from the stock antigen by dilution of the latter with physiological (0.85 percent) saline (C. P.) [5] solution containing 0.3 percent phenol (Merck's blue or silver label brand), to a turbidity corresponding to 0.75–1.00 on the McFarland nephelometer scale. The hydrogen-ion concentration of the dilute antigen is corrected to pH 8.2 to 8.5 by the addition of dilute sodium hydroxide. In this narrow pH range thymol blue used as indicator (5 or 6 drops of the standard stock dilution added to from 5 to 6 cc of the diluted antigen in ⅝-inch test tubes) imparts a dirty green to greenish-blue color to the tube. Cresol red (7.2 to 8.8) also may be employed. The color reaction should be checked frequently by comparison with standard buffer solutions, with the use of the comparator block, or by the potentiometer method. New dilute antigen should be prepared each day and kept cold.

The diluted antigen is employed in 2-cc quantities in 4-inch by ½-inch test tubes or 1-cc quantities in smaller tubes in which the final serum-antigen mix-

[4] The stock cultures are maintained by transferring to new sloped agar at least once a month and keeping at 18° to 25°C. (average room temperature) in a dark closet or chest, following incubation for from 24 to 36 hours at 37° C. The purity of the stock cultures should be checked at definite intervals by microscopic examination of Gram-stained slides.

[5] The sodium chloride should be of tested purity.

tures are made and incubated. The distribution of the antigen in the tubes may be accomplished by the use of long burettes, or special filling devices made for the purpose.

Serum dilutions, incubation, etc.—Either of two dilutions of the test sera may be employed, namely 1:25 or 1:50.[6] The 1:25 dilution, when used, shall serve as a finding dilution, and shall not be used to condemn a flock which has been placed on a * * * (pullorum-clean) list, or the immediate progeny of a * * * (pullorum-clean) flock, unless at autopsy the organism is found, or there is complete agglutination in dilutions of at least 1:50. In all official reports on the blood test the serum dilutions shall be indicated. The sera are introduced into the agglutination tubes in the desired amounts with well-cleaned serological pipettes or special serum-delivery devices which do not permit of the mixing of different sera.[7] The antigen and serum are well mixed before incubation.

Interpretation of results.—The results shall be recorded as:

N, or —, when the tube remains uniformly turbid, and there is no distinct settling of antigen.

P, or +, when there is a distinct clumping of the antigen, and the liquid between and above the agglutinated particles is clear. The reaction should be full 4+.

S, or ?, when the agglutination is only partial or incomplete (1+ to 3+). The reactions may be recorded as slightly suspicious, S or ?, or strongly suspicious, *S* or *?*.

M, or missing, when samples listed on the original record sheet are missing.

H, or hemolyzed, when the blood samples were hemolyzed and unsatisfactory for the test.

B, or broken, when the sample tubes are broken on arrival, and no serum is obtained.

Some allowance must always be made for differences in the sensitiveness of different antigens and different set-ups, and therefore a certain amount of independent intelligent judgment must be exercised at all times. Also the histories of the flocks require consideration.

ADDENDA

Preparation of the nutrient agar for antigen production.—Dissolve 20 grams of dry Difco agar in 1,000 cc of water, by heating for 15 to 20 minutes in a pressure sterilizer at 15 pounds' extra pressure. Add to the hot agar solution 4 grams of Difco beef extract and 10 grams of Difco Bacto-peptone. Test the reaction and adjust to as near pH 7.0 (6.8 to 7.2) as possible with dilute NaOH or HCl, as may be required. Heat for 10 to 15 minutes in the autoclave. Filter, if necessary, through absorbent cotton in funnel, and fill into large tubes, special flasks or bottles.[8] Sterilize by autoclaving for 20 to 25 minutes at 15 pounds' extra pressure.[9] If partly purified or refined agar (preferably granular) is employed, the hot liquid medium should filter readily through the cotton, and be ready for tubing. Clarification with egg white or whole egg is necessary only when a poor or crude grade of agar is used. Stock nutrient agar should be kept away from sunlight and dust, and in a cool place.

THE STAINED-ANTIGEN, RAPID, WHOLE-BLOOD TEST

(Schaffer, MacDonald, Hall, and Bunyea. Jour. Amer. Vet. Med. Assoc. 79 (n. s. 32): 236–240. 1931.)

NOTE.—The description of the preparation of the antigen is not herein reprinted because that product is produced only under license from the Secretary of Agriculture in accordance with specific directions.

The Loop Method.—The loop method is carried out as follows: At the end of a 2½-inch length of nichrome wire (Brown and Sharp gage no. 24) a

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[6] There is much difference of opinion whether 1:25 or 1:50 should be the minimum dilution, and considerable research must be done before a final agreement can be reached.

[7] Where serological pipettes (graduated in 1/100 cc) are employed, a separate pipette should be used for each serum. Care must be exercised at all times to prevent the laking of the red corpuscles with water.

[8] If the ingredients dissolve completely and the reaction needs no adjustment, the second heating and the filtering may be omitted.

[9] When Blake bottles or Kolle flasks are used for growing the antigen, an additional heating for 5 minutes is desirable.

loop 3/16 inch in diameter is made. For convenience the wire is inserted into a small cork stopper which serves as a handle. It has been found that the blood can be measured rapidly and with sufficient accuracy by use of the wire loop. Such a loop when filled with blood, so that the blood appears to bulge out, delivers 0.02 cc. The antigen is measured by means of a medicine dropper whose tip is adjusted to deliver 0.05 cc when operated in the vertical position. By this means, the proportion of antigen to blood is kept between the limits of 2 to 1 and 3 to 1, which has been found to give the most satisfactory results. A drop of stained antigen is placed on the glass plate. A loopful of blood is taken up from the wing vein. When submerged in the blood and then carefully withdrawn, the loop comes away properly filled. On looking down edgewise at the filled loop, one observes that the blood appears to bulge out. The loopful of blood is then stirred into the drop of antigen and the mixture spread to a diameter of about one inch. The loop is then rinsed in clean water and dried by touching it to a piece of clean blotting paper, if necessary.

The glass plate is rocked from side to side a few times to mix the antigen and blood thoroughly, and to facilitate agglutination. The reaction is usually visible in from 5 seconds to 2 minutes. Slight reactions, which require more than 2 minutes, should as a rule be disregarded.

Various degrees of reaction are observed in this as in other agglutination tests. The greater the agglutinating power of the blood the more rapid the clumping and the larger the clumps. A positive reaction consists of a clumping of the antigen in well-developed violet flocculi surrounded by clear spaces. This reaction is easily distinguished against a white background. A somewhat weaker reaction consists of small but still clearly visible clumps of antigen surrounded by spaces only partially clear. The interpretation of these partial reactions should be the same as that of similarly incomplete "tube method" agglutination reactions. Between this point and a negative reaction there sometimes occurs a very fine granulation barely visible to the naked eye; this should be disregarded in making a diagnosis.

The very fine marginal flocculation which may occur just before drying up is also regarded as negative. In a nonreactor the smear remains homogeneous. A glass plate about 15 inches square providing space for 48 tests has proved satisfactory for this work. The use of the plate enables the tester to have a number of successive test mixtures under observation without holding up the work to wait for results before proceeding to the next bird. As a result of more than a year of experience in testing with this antigen, it has been decided to regard as definitely positive only those reactions which appear within one minute after mixing the antigen and blood, while those which appear more slowly are regarded as suspicious.

THE RAPID SERUM TEST

(Runnells, Coop, Farley, and Thorp. Jour. Amer. Vet. Med. Assoc. 70 (n. s. 23): 660–662. 1927.)

Preparation of Antigen, Rapid Method.—One strain of *Bacterium pullorum* is grown on nutrient agar (pH 7.5) for 48 to 72 hours, washed off with a very small amount of a 12 percent solution of sodium chloride containing 0.5 percent phenol, filtered through glass wool to remove clumps of bacteria and of media and adjusted so that its turbidity is 50 times greater than tube 0.75 of McFarland's nephelometer.

Setting up Tests, Rapid Method.—The tests are run on glass, ruled off in inch squares. The glass forms the top of a box in which is a frosted electric-light bulb that furnishes light and heat. The interior of the box is painted black. Two serum-antigen dilutions are used for each sample. The amount of antigen in each case is 0.02 cc. This amount of the concentrated antigen is equivalent to 1 cc of the antigen diluted to correspond to tube 0.75 of McFarland's nephelometer. Since this is the amount and turbidity of the antigen employed in the slow method, the two tests are placed on the same basis as regards antigen. The serum used is in 0.02-cc and 0.01-cc amounts. Therefore, the serum-antigen dilutions are equivalent to 1–50 and 1–100 in the slow method. The serum and antigen are placed a short distance apart in the squares and are mixed thoroughly with a toothpick. With positive sera agglutination usually takes place immediately. Occasionally a serum-antigen mixture is found that requires nearly five minutes for complete flocculation to occur. When this kind occurs the reaction can be hastened by agitation with a toothpick.

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